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INTRODUCTION

Historically, heterotopic ossification (HO) and hypertrophic callus formation has been associated with traumatic brain injury (TBI), spinal cord injury and severe burns. Heterotopic ossification is a distinct skeletal complication (phenotype) in military and civilian trauma patients with high velocity fractures. Despite an incidence of 11-25% in multiply injured civilian patients and patients, and 63% in combat related amputations, the phenomenon is still poorly understood.[1-4]

Data suggests that bone remodeling is subject to significant autonomic control via beta-2 (ß2) adrenergic receptors on osteoblasts and involves the hormone leptin.[5,6] Patients with multiple trauma and/or brain injury frequently have autonomic nervous system dysfunction,[7,8] but the potential link to heterotopic bone formation has not yet been studied. Furthermore, not all patients with similar injury patterns and demographics develop HO, suggesting a genetic component, which under the appropriate environmental and physiologic conditions, predisposes some patients to the complication.

The process of heterotopic bone formation around fractures and ectopic bone around joints and soft tissue unrelated to trauma appear to be the same phenomenon.[9-11] This created a unique opportunity to prospectively monitor patients with traumatic fractures using serial radiographs for heterotopic bone formation and to match these with controls based on demographics and injuries. We have now examined 1095 patients over a two year period for HO and single nucleotide polymorphisms that may be associated with HO.

Certain individuals are genetically predisposed to complications which may be triggered by environmental influences.[12] While this phenomenon is often associated lung cancer and cigarette smoke, there is also evidence of genetic predisposition to common skeletal diseases such as osteoporosis.[13,14] We are beginning to identify genes that may predispose patients to HO should they have a traumatic injury to the musculoskeletal system.

Understanding the genetic factors underlying a complication allows stratification of "at risk" patients and the subsequent development of new therapy. Standard treatment regimens are not benign, and presently include the use of radiation therapy and anti-inflammatory medications, such as indomethacin. Patients with acetabular and long bone fractures given indomethacin for HO prophylaxis had a significantly higher rate of nonunion (26% versus 7%) than controls.[15] Radiation therapy has fewer side effects, however it must be administered within 72 hours of injury, limiting its use in a combat environment.[16-18] Consequently, our current therapeutic strategies have limitations and stratification of patients by risk of HO may have important implications for clinical care.

At the end of Year One, we have achieved most of our expected milestones. This has culminated in an abstract that has been accepted for a podium presentation at the Basic Science Forum at the Orthopaedic Trauma Association meeting in October 2009.

BODY

Aim 1: To examine the relationship of genetics to the Heterotopic Ossification (HO) phenotype.

Data from 1095 consecutive trauma patients over a 24 month period have thusfar been examined. These patients all have DNA samples in our genetics repository and radiographic records of fracture or amputation with a minimum of 8 weeks of radiographic follow up. Radiographic review demonstrated heterotopic bone formation in 108 (10%) of these patients. This is a percentage that is consistent with rates published in the literature for civilian populations. The anatomic subsets are as follows: Femur 29%; Pelvis 46%; Humerus 11%; Forearm 7%; Tibia 5%; Elbow 2%. The remaining patients without HO were used as the comparison group.

DNA was extracted from all patients using the PUREGENE system (Gentra Systems Inc., Minneapolis, MN). Sixty one single nucleotide polymorphisms were examined including genes associated with autonomic, metabolic and inflammatory pathways (see Appendix). Genotyping was done using 5' allelic discrimination assay (Applied Biosystems Inc., Foster City, CA) and mass spectroscopy (Sequenom Inc., San Diego, CA).

Sixty one SNPs were examined, and of these, 3 polymorphisms reached significance for association with HO: rs1042714 (a β_2-Adrenergic Receptor, ADRB2), rs4986791 (Toll-Like Receptor 4, TLR4), and rs4986791 (Complement Factor H, CFH). ADRB2 was associated with increased risk of developing HO, and TLR4 and CFH were associated with decreased risk. Interestingly, these are genes that are associated with three distinct physiologic pathways: 1. The adrenergic system, 2. Immune signaling, and 3. Inflammation. The SNPs associated with HO have been previously associated with non-skeletal trauma outcomes.

Gene, (SNP)	**Population freq of effect genotype**	**Pathway**	**Odds Ratio, (95% CI)**	**p-value**
CFH, (CC vs. TT)	39.0%	Complement Activation	0.52 (0.29 - 0.94)	0.03
ADRB2, (CC vs GG)	15.7%	Adrenergic Nervous System	1.86 (1.05 - 3.29)	0.03
TLR4, (CC vs. CT)	10.2%	Innate immunity activation	0.42 (0.16 - 1.00)	0.05

Accrual and genetic extraction are ongoing. At present we have extracted DNA from 3000 specimens already have 2000 more in our repository awaiting extraction. Data from these patients will be added to the present data on a continual basis.

Aim 2: To determine the environmental effect (ie, injury severity, traumatic brain injury, medications) on phenotype expression.

Data from the trauma registry (TRACS) is being compiled to include associated injuries (head, chest, abdomen, etc) as well as information on medications, pre-existing medical conditions, and demographics from the electronic medical record for the current cohort of patients. This is being de-identified as we compile the data. We will begin statistical analysis to determine gene-environment interactions once we identify at least 200 HO patients to provide power to the statistics. We expect to be able to do this starting in month 18 as planned.

Aim 3: To determine clinical biomarkers which predict the HO phenotype.
Data capture has already been performed on the patients in our current cohort. We will compile this data with environmental data in Aim 2 as we complete genotyping.

KEY RESEARCH ACCOMPLISHMENTS

- Reviewed 1095 radiographs for presence of HO in a trauma population
- Identified 108 patients with the HO phenotype
- DNA extraction performed on these patients
- Examination of 61 SNPs in this population for inflammatory, immune and bone markers
- Found statistical significance for 3 SNPs (CFH, TRL4 and ADBR2) in association with formation or protection from HO
- Data accepted for presentation at the Annual Orthopaedic Trauma Association Basic Science Forum

REPORTABLE OUTCOMES

We have demonstrated an association between three polymorphisms and heterotopic ossification. TLR4, CFH and AD2BR are polymorphisms that have been noted in prior studies for their association with morbidity in trauma. These genes mediate physiologic processes that are not yet directly linked bone remodeling.

We have been accepted for podium presentation of our early findings at the Basic Science Forum at the Orthopaedic Trauma Association annual meeting in October 2009. This abstract is attached in the Appendices.

We have submitted a grant proposal to NIAMS/NIH in response to the GO Grant Opportunity to build on this research as we identify more SNPs, and increase our sample size.

CONCLUSION

We have identified three polymorphic variations that are associated with the development of heterotopic ossification. These include SNPs that are known to be involved in the inflammatory process (TRL4 and CFH) and autonomic regulation (β_2 Adrenergic Receptor). In addition, these SNPs have shown significance in other studies associating these genotypes with morbidity and mortality after trauma. This early data adds evidence to the theory that there is a mechanism of central nervous system control of bone remodeling through autonomic and inflammatory processes. Heterotopic ossification may be related to autonomic dysregulation secondary to genetic predisposition and/or head injury. This study provides evidence for at least three distinct pathways associated with HO, the adrenergic system, immune signaling and the alternative complement system. Over the next two years, we will add more specimens in the analysis, and also include more candidate genes. We will also begin to add the clinical data including

associated injuries and physiologic markers such as heart rate variability to determine clinical markers of these genetic differences that may relate to the systemic response to trauma in certain individuals and that may also affect the remodeling of bony injury. Furthermore, we are seeking funding to expand this project to a genome wide association study for a more thorough understanding of the genetic influences and interactions in bone remodeling.

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The Genetics of Heterotopic Ossification: Insight into the Bone Remodeling Pathway

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PURPOSE: Heterotopic ossification (HO) occurs in 12-20% of civilian and 63% of military fracture patients resulting in significant morbidity (pain, reduced function, re-operation). Others have demonstrated bone remodeling following fracture is regulated by numerous physiologic pathways (inflammation, autonomic nervous system, glucose metabolism) and environmental conditions (open fracture, wound classification, traumatic brain injury, and medications). This study presents the first association between HO and the human genome. **Hypothesis**: Genetic variation in multiple physiologic pathways modifies bone remodeling and in some individuals increases their risk of HO after fracture.

METHODS: Over 24 months, 1095 consecutive trauma patients were admitted to the ICU, DNA obtained, and subsequent radiographs reviewed for HO. 108 patients (10%) were identified with HO and compared to 982 non-HO patients. DNA was extracted from the 1095 patients using the PUREGENE system. (Gentra Systems Inc., Minneapolis, MN.) 61 SNPs representing a variety of structural, metabolic and autonomic pathways were examined. Genotyping was done via 5' allelic discrimination assay (Applied Biosystems Inc., Foster City, CA) and mass spectroscopy (Sequenom Inc., San Diego, CA). Multivariate logistic regression was used to analyze each SNP independently while adjusting for severity of injury (as measured by TRISS).

RESULTS: Of 61 SNPs, 3 polymorphisms reached significance for association with HO: rs1042714 (a β_2-Adrenergic Receptor, ADRB2), rs4986791 (Toll-Like Receptor 4, TLR4), and rs4986791 (Complement Factor H, CFH). ADRB2 was associated with increased risk of developing HO, and TLR4 and CFH were associated with decreased risk.

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CONCLUSIONS: The genome may affect bone remodeling after fracture, in some cases resulting in heterotopic ossification.

1) Three distinct pathways were associated with risk for HO: the adrenergic system, immune signaling and the alternative complement system

2) This preliminary data reinforces previous observations linking bone remodeling to autonomic and inflammatory processes.

3) This is an association study, requiring validation in a separate and equivalent patient population. This work is underway.

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